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(S3)

(71) Applicant
Aluminum Company of America

(Incorporated in the USA - Pennsylvania)

Alcoa Building, Pittsburgh, Pennsylvania, United States of America

(72) Inventors
Larry F Wieserman
David H DeYoung
George M Whitesides

(74) Agent and/or Address for Service
Langner Parry
High Holborn House, 52-54 High Holborn, London, WC1V 6RR, United Kingdom

BAN

(54) Biologically reactive particles with biological, therapeutic and chromatographic applications

(57) The particles comprise (a) a metal oxide/hydroxide core to which is bound (b) functionalized organophosphorus compound such that the P-containing groups are bound to the active sites of the core and which forms a monomolecular layer with the functionalized C-containing groups oriented away from the core surface and (c) biologically active ligand bound to the exposed functional groups. The ligand may comprise a whole cell or an antibody, antigen, hapten, enzyme particularly protein A) or substrate or inhibitor thereof, co factor, binding or carrier protein or compound bound thereto, hormone, receptor, repressor, inducer, oligonucleotide, nucleic acid, amino acid, saccharide, dye (particularly Cibacron Blue), polyacrylamide, latex or ion-exchange material. The particles are used to immobilise enzymes or single strand DNA/RNA, in cell sorting and immunoassay, in the treatment of disease, and or packing material in chromatographic methods.

BIOLOGICALLY REACTIVE PARTICLES + COMPRISING LIGAND LINKED TO METAL OXIDE OR HYDROXIDE THROUGH ORGANOPHOSPHORUS CPD.

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At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

This print takes account of replacement documents submitted after the date of filing to enable the application to comply with the formal requirements of the Patents Rules 1982.

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FIG. 1

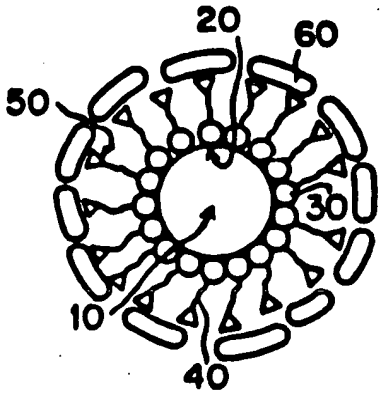
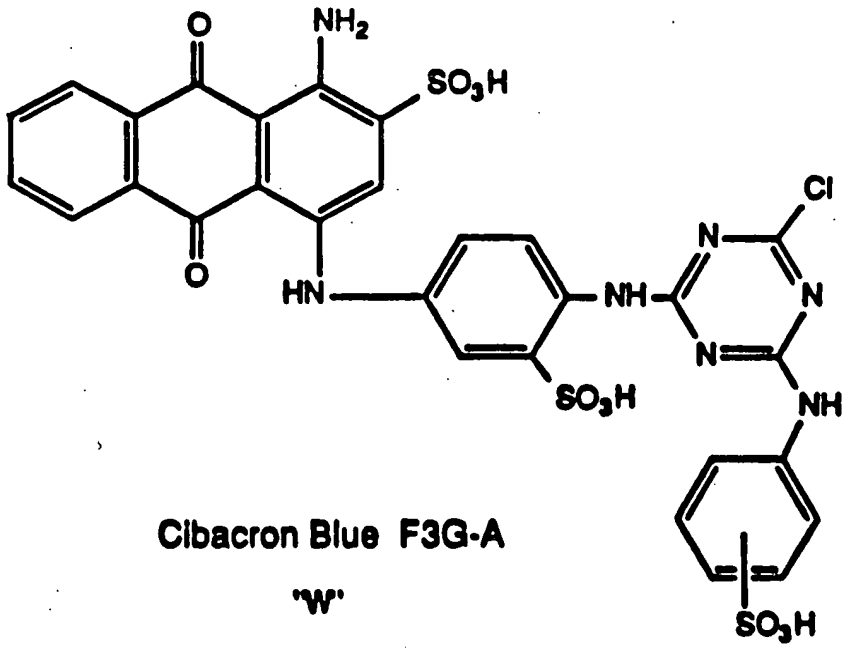


FIG. 2



Cibacron Blue F3G-A

"W"

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BIOLOGICALLY REACTIVE PARTICLES FOR USE IN BIOLOGIC AND CHROMATOGRAPHIC APPLICATIONS

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1. INTRODUCTION

This invention relates to modified metal oxide particles coupled to biologically active materials. More particularly, this invention relates to the production of such complexes and their use in a variety of applications. Such complexes, it has been found, are useful in an array of procedures including, but not limited to, biological assays, immobilized enzyme reaction systems, chromatography, affinity absorption, nucleic acid hybridization, and cell separation techniques.

2. BACKGROUND OF INVENTION

2.1. ORGANIC AND INORGANIC ADSORBENTS

Analytical and industrial adsorbents made from organic resins suffer from poor physical strength, poor thermal stability, high cost, solvent swelling, and low capacity. Adsorbents made from metal oxides such as silica exhibit poor chemical stability at high pH. For many applications, in particular those requiring high pressure or large-scale separations, an adsorbent with high physical integrity, good chemical stability over a wide range of pH conditions, specific surface functionalities, good thermal stability, and low cost is needed for a wide range of applications.

Other metal oxides such as alumina have also been used as adsorbents because of the good physical integrity and low cost of alumina. The solubility of alumina in pH ranges between 4 and 9 is very low and the material is, therefore, chemically and physically stable in this pH range. However, beyond this pH range, on either the basic or acidic side, alumina becomes soluble in aqueous media and its physical strength and integrity degrades rapidly.

Modifications of metal oxide adsorbents such as alumina and aluminosilicates have been proposed. Stockel U.S. Patent 4,506,628 teaches the formation of an adsorbent animal litter utilizing alumina, aluminosilicates, or coal residues as the

substrate intimately mixed with monomers containing acid functionalities which polymerize in situ. The monomer, such as vinyl phosphonic acid, together with a redox catalyst, is mixed with a pliable dough formed from alumina and water and extruded into pellets which harden as the monomer polymerizes.

Modified alumina has also been used in the formation of catalysts. Johnson et al. U.S. Patents 4,202,798 and 4,251,350 describe the formation of a hydrocarbon hydrotreating catalyst formed by contacting alumina with a phosphorus-containing acid compound such as phenylphosphonic acid and then calcining the phosphorus-containing hydrous alumina. The calcined alumina is then treated with at least one metal-containing compound and again calcined to form the catalyst product.

In addition, Cupery U.S. Patent 3,013,904 discloses a substrate having an organic polymer containing pentavalent phosphorus bonded thereto. Coatings of phosphorus-containing organic polymers are applied over coatings of positively charged colloidal metal oxides applied to negatively charged substrates. The thickness of the combined colloidal oxide and polymer layers on a substrate is less than 100 millimicrons.

Venables et al. U.S. Patent 4,308,079 describes the treatment of an aluminum oxide surface of an aluminum substrate with a monomolecular layer of an amino phosphonate compound such as nitrilotris(methylenetriphosphonic acid) to retard hydration of the aluminum oxide to aluminum hydroxide to provide a more stable microporous surface which is particularly suited to commercial adhesives. The presence of the hydrated oxide is said to interfere with the formation of a satisfactory bond between the adhesive and the oxide, while the phosphonate treatment is said to inhibit the conversion of the oxide to hydroxide without interfering with subsequent bonding of the adhesive to the oxide.

2.2. BIOLOGICAL SEPARATION SYSTEMS

A wide variety of biologically-related applications requires the coupling of a biologically active molecule or complex to an inert support. For example radioimmunoassays (RIA) and enzyme-linked immunosorbent assays (ELISA) require that the labeled antibody be attached to a support such as a coated tube (see U.S. Pat. No. 3,646,346), a particle such as a glass bead (see U.S. Patent Nos. 3,652,762 and 3,555,143), or a magnetically active particle (see U.S. Patent No. 4,672,040). In each of these procedures, the support aids in the separation of bound and unbound material, e.g. the antigen-antibody complex, by virtue of its attachment to the support, can be removed from solution by physical processes such as centrifugation. This procedure aids in both the separation and assay of biological substrates.

Similarly, enzymes used in industrial reaction systems are preferably bound to a stationary support to prevent their removal when the reaction products are isolated. Such supports can be comprised of an array of materials including glass, ceramics, and metals.

For a material to be useful as a support it must be inert to the biological material and stable under conditions likely to obtain during the procedures. It is often also advantageous to choose supports having a particle size which is sufficiently small to permit the easy dispersion and allow efficient mixing of the bound biological material in the reaction or assay medium. Additionally, the coupling effected between the biologically active material and the support must be of sufficient strength to prevent separation during the desired application. Thus, a material or class of materials meeting these criteria is needed.

3. SUMMARY OF INVENTION

It is an object of this invention to provide organophosphorus-modified metal oxides coupled to biologically active materials for use in a variety of applications. It is further an object of this invention to provide such complexes which are sufficiently stable to resist the extreme conditions normally found *in vivo* and during *in vitro* biological assays and other procedures.

This invention provides complexes of metal oxide organophosphorus compounds coupled to biologically active materials. The coupling takes place via the functionalized organic group of the organophosphonate or organophosphinate intermediate and can be either direct or through the intermediacy of a conjugative group. Biologically active materials are defined herein as compounds or molecules, including macromolecules, which exhibit a biological, physiological activity and/or function. Some examples include, but are not limited to, antibodies, antigens, haptens, enzymes, apoenzymes, enzymatic substrates, enzymatic inhibitors, cofactors, binding proteins, carrier proteins, metalloproteins, compounds bound to these proteins, lectins, mono-, oligo-, and polysaccharides, hormones, receptors, repressors, inducers, nucleic acids, dyes, amino acids, oligonucleotides, DNA or RNA fragments. Other natural and synthetic materials such as polyacrylamides, latex, agarose, dextran, and ion-exchange materials and related resins are encompassed by the present definition.

In this way, a large array of biologically active materials, including whole cells, yeast, and bacteria can be coupled to the particles. The resultant complexes can then be useful for a wide variety of applications which include but are not limited to bioactivity assays, immobilization systems, chromatography and separation techniques, high-pressure applications, affinity absorption, and nucleic acid hybridization and replication.

The particles themselves, termed metal oxides, are more properly termed metal oxides/hydroxides and include a broad spectrum of compounds ranging from the pure oxides to the pure hydroxides, and encompassing all oxide/hydroxide mixtures therebetween. The metal useful in these particles include, but are not limited to, the alkali and rare earth metals as well as the transition metals and metalloids. Each particle can be comprised of a metal or combinations of two or more different metals. By proper choice of manufacturing conditions, the overall particle size can be varied. In a preferred embodiment of this invention, aluminum oxide/hydroxides are used.

These metal oxides are reacted with functionalized phosphonic or phosphinic acids, preferably in aqueous media, to form the corresponding phosphonate- or phosphinate-derivatized metal oxide particle. A wide array of acids can be used, but those organophosphorus compounds containing 1-60 carbon atoms are preferred. In addition, the monoesters and diesters of phosphoric acid may be employed.

A wide variety of biologically active ligands can then be attached to these organophosphorus compounds. Many methods can be used to effect this attachment including carbodiimide coupling (via carboxy termini) or glutaraldehyde coupling (via amino termini).

Additionally, compounds not amenable to such treatment can be coupled via an affinity binding method. For example the direct coupling of a whole cell to the particle will be quite difficult due to the severe strains induced by chemical coupling. However, the cell can be complexed with an antibody specific for certain antigenic determinants on the cell. Thus, coupling can be achieved by complexing the cell with an antibody which is, in turn, attached to the organophosphorus moiety.

Binding of the biologically active ligand to the functionalized carbon-containing group of the organophosphorus compound is also accomplished by noncovalent means such as ionic, electrostatic, magnetic interactions, van der Waals forces, or hydrogen bonding.

4. BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the biologically reactive particle of the present invention: the metal oxide/hydroxide core is indicated by 10; the surface active sites are shown at 20; the organophosphorus compound is 40 with its phosphorus containing group at 30 and the functional group, 50; the biologically active ligand is 60.

Figure 2 shows the structure of Cibacron Blue F3G-A or compound W.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1. METAL OXIDE ORGANOPHOSPHORUS COMPLEXES

In accordance with the invention, the derivatized or modified material comprises a metal oxide/hydroxide surface having chemically bonded thereto a substantially monomolecular layer of an organophosphorus compound wherein the organic portion is further comprised of a functionalized carbon-containing group oriented away from the surface of the metal oxide/hydroxide particle and capable of functioning as the active component of the monolayer. This "functional or active" material is suitable for use as an adsorbent which is stable over a large pH range, i.e., from 1-14 and which, unlike at least some of the metal oxide/hydroxide support material, is insoluble in aqueous media.

The use of the term "active material" is intended to define an organic molecule comprising a monomer, oligomer or short chain polymer having a phosphorus-containing group, preferably at one end of the molecule, capable of bonding to the metal oxide/hydroxide support and having one or more

additional functional sites thereon, preferably at the opposite end of the molecule, which may be used for the coupling, bonding, or adsorbing, etc. of atoms, ions or other molecules thereto. For example, when the active material functions as an adsorbent, it will have sites available onto which the material to be adsorbed will be attracted.

The use of the term "metal oxide/hydroxides" herein is intended to define a broad spectrum of oxides ranging from those which may contain few hydroxides, e.g., activated or calcined forms of aluminum oxide (metal oxide), to more hydrated forms which may comprise mainly hydroxide, e.g., $Al(OH)_3$. It has been found, however, that the metal hydroxide form, rather than the metal oxide form, provides a better bond with the phosphorus-containing group of the organophosphorus molecule with which it is reacted. However, for certain applications, dehydrated or activated forms of the metal oxide/hydroxide may be preferred due to the higher total surface area of such particles. For example, when aluminum oxide/hydroxide is used, the hydrated form, e.g., gibbsite, bayerite, or boehmite, will be preferred when only a large external surface area is desired; activated metal oxide will be preferred when it is desirous that the metal oxide/hydroxide have a high internal surface area as well.

As presently understood, the metal oxide/hydroxides suitable for use in the present invention usually require hydroxyl groups on the surface thereof for purposes of providing bonding sites for the phosphorus-containing organic materials. These surface hydroxyl groups react with the phosphorus-containing group or groups of the organic molecule, i.e., with the $-PO(OH)_2$ or -POOH acid group(s) of the phosphonic or phosphinic acid, respectively. When using an organic solvent, particularly where the solvent is immiscible with water, a surface of hydroxyl groups is provided on the metal oxide particles for purposes of reacting to form a chemical bond with the phosphonic or phosphinic acid groups.

It will be appreciated that as more and more of the surface hydroxyl units react with the organophosphorus compounds, the remaining hydroxyl sites become less and less accessible, especially where the organic group of the organophosphorus compound is bulky. Thus, it may be desirable, but not necessary, to allow the derivatized metal oxide/hydroxide to react with a second smaller, less bulky organophosphorus compound, e.g., methyl phosphonic acid, to "cap" any remaining free hydroxyl sites.

In addition, organophosphorus compounds having two or more phosporus-containing groups may be employed to provide a firmer bonding with the metal oxide/hydroxide surface.

Metal oxide/hydroxides which may be used as the support particle for reaction with the phosphorus-containing organic material include any metal capable of forming oxide/hydroxide selected from the class consisting of groups IB, IIA, IIB, IIIA, VA, VB, IIIB, IVA, VIB, VII and VIII, and combinations thereof. For example, the oxide/hydroxides of aluminum, magnesium, titanium, zirconium, iron, silicon, chromium, zinc, vanadium, and combinations of these may be used. Also, a core or center of iron oxide/hydroxide or other paramagnetic or ferromagnetic material may be used with a coating of a metal compound to take advantage of the magnetic properties of the iron oxide/hydroxide core. It should be noted that by use of the term "metal," it is intended to include not only the traditional metals, but also materials sometimes referred to as metalloids such as Si, Se, B, As and Te and to exclude the remaining nonmetallic elements of the periodic table. In addition, oxides/ hydroxides of the lanthanide series, as well as oxides/ hydroxides of thorium and uranium in the actinide series, may be used, if desired, as the support particle.

With respect to the metal oxide/hydroxides used in the resent invention, it is preferred that they be provided in particulate form for certain applications. As adsorbents, particle sizes can range from as low as 50 Angstroms to

provide large external surfaces and up to 12 millimeters for large reactors. When the adsorbent base is metal oxide the particle size can be 1 to 200 microns. It will be appreciated that other uses, for example, flocculation, flame retardation of polymers, heterogeneous catalysis and other uses described in this specification, can require different particle sizes. However, normally for adsorbent use, the particle size is greater than 1 micron. Typical particle size distributions, when the metal oxide/hydroxide particles comprise aluminum oxide/hydroxide are 0.5-2, 3-6, 7-12, 10-18, 18-32, 32-63, and 50-200 microns.

With respect to the particle morphology of the metal oxide/hydroxides used in the invention, e.g., metal oxide/hydroxides such as metal oxide, or iron oxide, for purposes of the present invention, it is preferred that they have a pore size of 20 Angstroms to about 100 microns in diameter. Further, it is preferred that the particles have a pore volume of 0.1 to 1.5 mL/g. The level of impurity should be minimized depending on the end use. However, for adsorbents, for example, the metal oxide or metal compound should have a purity level of over 80%, preferably 95% or greater. Surface area of the particle is preferred to be high with typical surface areas for metal oxide, for example, being in the range of 0.10 to 600 m^2/g and up to 1000 m^2/g for other metal oxide/hydroxides such as silica.

To produce the active material comprising the metal oxide/hydroxide modified with one or more types of phosphorus-containing organic molecules, the metal oxide/hydroxide, is allowed to react with a phosphonic or phosphinic acid, or mixtures thereof, in an aqueous media at a temperature of from about 25°C up to about 100°C, preferably about 50°C, for a period of from at least 0.1 to not more than 20 hours, and preferably from at least about 0.5 hours up to about 4 hours, using an initial acid concentration of at least about 0.0001 to 0.1 molar. In some instances higher

concentrations may be desired. When the media is nonaqueous, the temperature range can be greatly extended. For example, it may range from 5°C or lower to 200°C or sometimes higher depending on the liquid medium.

In the present invention, the weight of the monolayer can range from about 0.01 to 90 percent by weight of the total weight of the coated metal oxide, and preferably the monolayer can range from about 4 to 50 wt%, typically 5 to 20 wt%. When the metal oxide/hydroxide particle is very small, the monolayer weight can be equal to or greater than the weight of the particle. For purposes of ensuring that a monolayer of organophosphorus material is bonded to the metal oxide particles, the application should be carefully controlled. That is, a monolayer can be obtained, for example, by controlling the viscosity of the aqueous media, the time of exposure in the media, the concentration of phosphorus-containing organic material in the media, or the concentration of hydroxyl units on the surface of the metal oxide particles. In addition, after formation of the active material, it may be washed with an organic solvent, a basic or acidic solution, or combinations thereof. Treatment, for example, with a $NaHCO_3$/Na_2CO_3 washing solution, having a pH of about 10, removes weakly adsorbed molecules on the particles. This procedure ensures that all of the molecules remaining are bonded to the hydroxyl groups on the metal oxide/hydroxide surface and not to one another, thus assuring formation of the desired monomolecular layer stable over a wide range of pH.

The formula for the phosphonic acid useful in the practice of the invention may be written as $RPO(OH)_2$ while the phosphinic acid may be written as RR'PO(OH) where R' may be hydrogen and both R and R' may each be comprised of 1-60, preferably 5-30, carbon-containing molecules such as an alkyl group. Mono- and diesters of phosphoric acid may also be employed. These compounds have the general formula (RO)(R'O)POOH where the R and R' carry the same meaning as

above. However, the resulting modified particles are stable in a narrower range of pH. Nevertheless, they are quite stable under physiological conditions. Other examples of groups which may wholly or partly comprise R and/or R' include long and short chain aliphatic hydrocarbons, aromatic hydrocarbons, carboxylic acids, aldehydes, ketones, amines, amides, thioamides, imides, lactams, anilines, pyridines, piperidines, anhydrides, carbohydrates, thiocyanates, esters, lactones, ethers, olefins, alkynes, alcohols, nitriles, oximes, organosilanes, sulfur-containing organic compounds, ureas, thioureas, perfluoro organic molecules, perchloro organic molecules, perbromo organic molecules and combinations of these groups.

The functionalized carbon-containing organophosphorus molecules such as those listed above may also include one or more inorganic groups substituted thereon such as halogens, nitrates, phosphates, phosphinates, phosphinites, phosphonates, quaternary ammonium salts, and the like. While it is preferred that the free end of the organic group extends away from the surface of the metal compound particle, it is within the scope of the present invention to provide, on the free end of the molecule, one or more functional groups. A functional group may be defined as the group on the molecule which enables the active material (comprising the phosphorus-containing organic material bonded to the metal oxide/hydroxide surface) to react with, attract, couple to, bond with, etc. other atoms, ions and/or molecules. Intermediate groups may be defined as the groups on the molecule which permit substitution or addition of groups or compounds to the R or R' groups after the monolayer has been formed on the oxide/hydroxide particle. Examples of intermediate groups include I, Cl, Br, CN, etc. The intermediate group permits the addition of groups or radicals which would not be compatible with or be destroyed during formation of the monolayer. Thus, this strategy permits the addition of

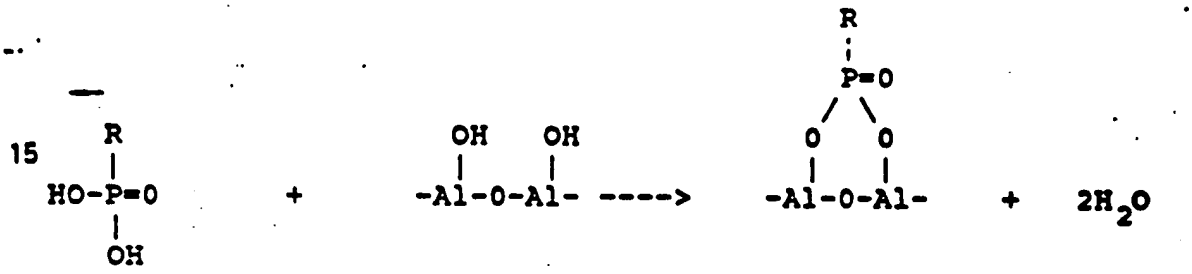
functional groups on the end of the R or R' group after the monolayer is formed. By attaching specific functional groups, either organic or inorganic, to the R and R' groups of the phosphonic and phosphinic acids, a wide variety of adsorbent selectivities, capacities, and other uses are provided.

The functional groups attached to or contained within the free end can be selected from cation exchange functional groups and anion exchange functional groups such as $-HSO_3$, $-N(CH_3)_3$ Cl, -COONa, $-NH_2$ and -CN, for example. The $-HSO_3$ functional group permits removal of cations such as Cu^{+2}, Fe^{+2}, Co^{+2}, Cd^{+2}, Ca^{+2}, Sr^{+2}, Hg^{+2}, Pb^{+2}, Ba^{+2} and Be^{+2} from aqueous media. The functional group, $-CH_2N^+(CH_3)_3Cl^-$ permits removal of anions such as HSO_4, ClO_3, NO_3, NO_2, HPO_4, formate, and citrate. Other examples of pendant functional groups include the following although some of these molecules also belong to the types of biologically active ligands to be bound to the organophosphorus moiety: a carboxyl group, a carboxymethyl group, a glucoside group, a monoclonal antibody, a cyano group (-C≡N), a phenyl group, a diphenyl group, a tertiary butyl group, a sulfonic group, a benzyl sulfonic group, protein groups such as protein A (staphylococcal protein A), protein G, immunoglobulins and binders thereof, pharmaceutical compounds, yeasts, microbes, whole cells, enzyme groups, dye molecules, chelated metal groups, tag molecules and combination of these groups. Further, it should be noted that the carbon group can be a saturated or unsaturated cyclic or a cyclic carbon chain. As mentioned previously, more than one functional group may be present within the carbon-containing group of the organophosphorus compounds.

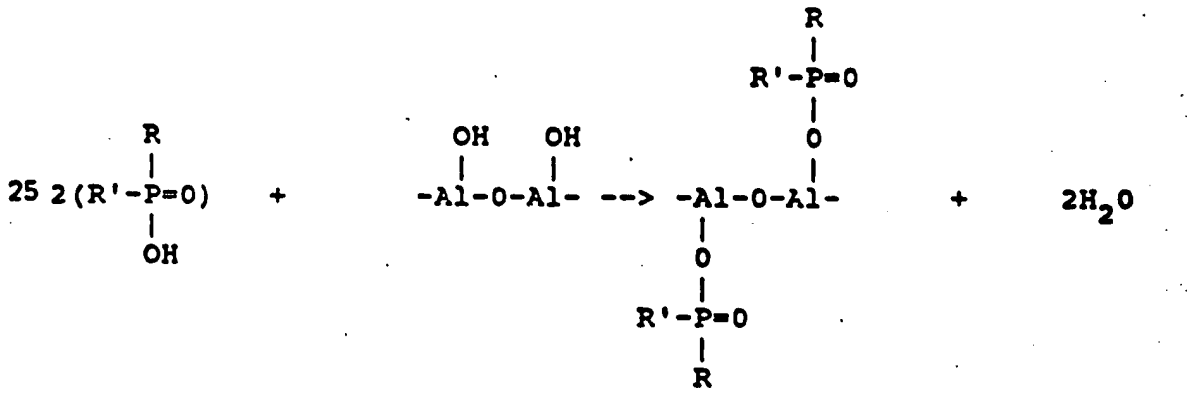
It will be noted that the R or R' groups are always monomers or oligomers. Preferably, the oligomers have a molecular weight of less than 2000. By the use of monomer herein is meant to include a chemical compound that can under-

go polymerization. By oligomer is meant a polymer or polymer intermediate containing relatively few structural units, i.e., a polymer containing 2-4 monomers.

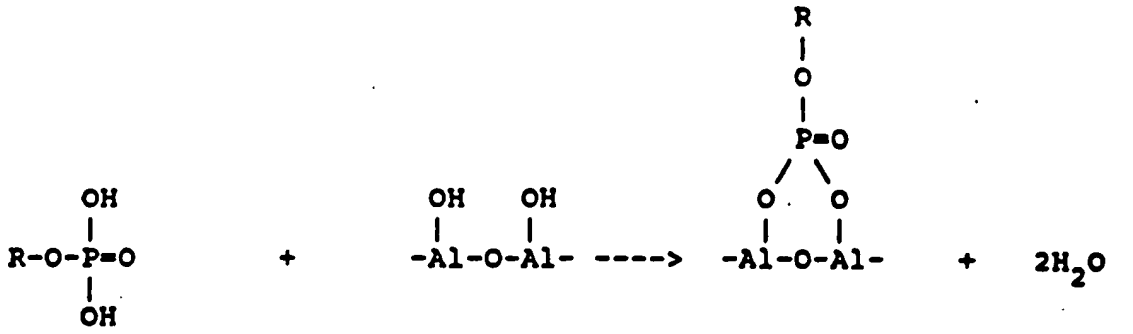
While we do not wish to be bound by any particular theory of bonding, it is believed that when a metal oxide/hydroxide particle, for example, metal oxide, is brought into contact with the phosphonic or phosphinic acid a reaction or adsorption of the acid on the alumina takes place in which the aluminum and phosphorus atoms in the respective molecules are apparently bonded together through an oxygen atom as illustrated in the formulas below:



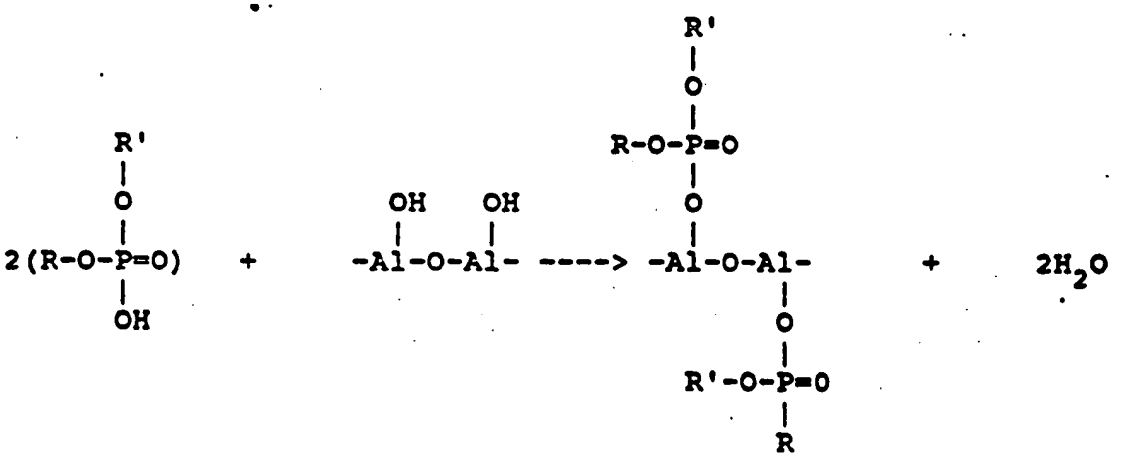
Phosphonic Acid + Hydrated Alumina → Product + Water



Phosphinic Acid + Hydrated Alumina → Product + Water



Phosphoric Acid Monoester + Hydrated Alumina → Product + Water



Phosphoric Acid Diester + Hydrated Alumina → Product + Water

Aluminum atoms may be six- or four-coordinate with or without coordination vacancies at or near the surface (external or within pore structures).

Thus it can be seen, using the above proposed model, that if all of the exposed hydroxyl groups on the surface of the metal oxide can be reacted with the phosphonic or phosphinic acid groups, the surface chemistry of the reacted metal oxide will change. Furthermore, the type of organic radical attached to the phosphonic or phosphinic acid can be tailored to achieve certain types of affinities to provide selectivity

in the adsorption characteristics of the product. For example, when a metal oxide treated with a phosphonic or phosphinic acid having an octadecyl R group is used, the chromatographic separation of a mixture of relatively nonpolar compounds can be achieved. For example, a mixture of p-nitroaniline, methyl benzoate, phenetole, and o-xylene under appropriate chromatographic conditions can be selectively adsorbed on such a reactive material in accordance with the present invention.

The chemical bonding of the phosphonic or phosphinic acid to the metal oxide/hydroxide particle, e.g. metal oxide particle, is illustrated in Figure 1 wherein the center 10 represents the metal oxide particle having a surface 20. The carbon-containing molecule is chemically bonded at one end to surface 20 by means of its phosphorus-containing group 30 and through a phosphorus-oxygen-metal bond. As presently understood, it is preferred that the opposite or free end of the carbon-containing molecule 40 extends away from the surface of the particle, as shown in Figure 1. Further, it is important to control the application or bonding of the phosphonic or phosphinic acid to the metal oxide/hydroxide support so as to obtain a monolayer bonded to the metal oxide/hydroxide particle as illustrated in Figure 1.

By "monolayer" or "monomolecular layer" is meant that 90%, and preferably 98%, of the phosphonic or phosphinic acid molecules are bonded to the metal oxide/hydroxide particle as a single layer of molecules. Thus the application should be controlled so as to prevent the R or R' groups from bonding to each other to form weakly adsorbed multilayers which might provide further hydroxyl units, i.e., -POOH units directed away from and not bonded to the surface of the metal oxide/hydroxide particles, thereby defeating the purpose of the invention, for example, when it is used as an adsorbent. The thickness of the phosphorus-containing bonded organic monolayer is in the range of 10-5000 Angstroms and preferably

20 to 500 Angstroms. Additionally, monomer or oligomer comprising the monolayer may have reactive sites which can permit crosslinking so as to polymerize monomers or oligomers already bonded to the surface of the metal oxide particle.

5.2. USE OF METAL OXIDE ORGANOPHOSPHORUS COMPOUNDS AS COUPLING AGENTS

As shown in Figure 1, the metal oxide organophosphorus compounds, by virtue of their attached organofunctionalities 50 can serve as a base to which various biologically active ligands 60 such as proteins, antibodies, hormones, pharmaceuticals, and other materials can be coupled. A variety of sulfonated polyaromatic, triazine, naphthalene, and anthraquinone dyes may also be coupled to the organophosphorus compounds. In a preferred embodiment, the dye whose structure is illustrated in Figure 2 (compound W) is used. The resulting biologically reactive particles can serve as intermediates for the affinity binding of cells, bacteria, yeasts, and other microorganisms. Such binding permits the use of the aforementioned materials in a large array of biological applications.

5.2.1. DIRECT COUPLING OF BIOLOGICALLY ACTIVE LIGANDS

The attachment of biologically active ligands to the organophosphorus compounds is dependent both upon the nature of the organofunctionality and the nature of the ligand to be attached. The precise means of the attachment will be governed by methods which can effect covalent coupling between their respective functionalities, and, also, which do not involve conditions sufficiently severe to denature or otherwise inactivate the biologically active ligand.

A wide variety of reactions fit these criteria, and can be used to form the coupled materials of this invention. For example, an organophosphorus compound containing a pendant carboxy group, i.e., such as a carboxylic acid at the free

end, can be attached to the metal oxide/hydroxide of the present invention. Biologically active ligands containing terminal amino groups, such as proteins and immunoglobulins, can then be attached to this pendant group through a carbodiimide intermediate. In such a process, a reagent, such as 3-(3-dimethylamino)propyl-1-ethylcarbodiimide, is mixed with the modified particles containing terminal carboxy functionalities; the bonding readily occurs at room temperature or below. The desired biologically active ligand is then mixed and allowed to react with the carboxy-carbodiimide adduct produced from the previous step. An amide bond is obtained at ambient temperature.

Alternatively, biological ligands containing terminal carboxy groups may first be allowed to react with the carbodiimide reagent. The metal oxide particles modified with organophosphorus groups which also contain terminal amino groups at the opposite end of the organic chain can then bind the biological ligands covalently. Two molecules containing pendant amines may also be joined by the intermediacy of a conjugative moiety such as glutaraldehyde. The resulting imine functionalities can then be reduced under very mild conditions with sodium cyanoborohydride.

Also, the organofunctionalities themselves can be modified directly to form groups more amenable to binding particular biologically active ligands. For example diazo derivatives of aromatic amines can be prepared by diazotization of the amine with nitrous acid directly. For organic compounds that contain terminal hydroxyl groups, treatment with p-nitrobenzoyl chloride and hydrogenation provides the terminal aromatic amine. The addition of nitrous acid as mentioned above yields the highly reactive diazonium salt. A number of macromolecules containing aromatic end groups, e.g., tyrosine or histidine, can thus be covalently bound to the functionalized organophosphorus group at temperatures close to ambient.

United States Patent No. 3,652,761, incorporated herein by reference, presents a large array of coupling chemistries useful for attaching biologically active compounds to organosilane compounds. Such methods are also effective for coupling biologically active ligands to the organophosphorus compounds of the present invention.

5.2.2. AFFINITY BINDING OF BIOLOGICALLY ACTIVE COMPOUNDS

Often, compounds are incapable of being covalently bound to the organofunctionalities either because the strains induced by bonding or the conditions required to effect the bonding will cause the compounds to denature. Additionally, effecting such bonding with large structures, such as whole cells, is often difficult since the surface chemistry is not sufficiently uniform to effect a strong bond.

In such cases, the compounds can be attached to the metal oxide substrates through intermediates which possess an active sites that have an affinity for, and are capable of binding, the compound. For example, any ligate can be bound to the modified metal oxide substrate by use of an antibody or lectin intermediate which is specific to that ligate. In such a procedure, the antibody is covalently attached to the metal oxide by any of the methods described in section 5.2.1. The ligate is then mixed with the antibody/metal oxide complexes, and the antibody acts to bind the specific ligate.

Alternatively, the antibody itself can be bound to the metal oxide via an intermediate protein molecule which can bind the antibody in a complex. The antibody can then bind the specific ligate. In such a system, an extremely useful compound for use as an intermediate protein is Protein A, which can bind tightly to the Fc region of immunoglobulins, leaving the active sites free.

Such a procedure is particularly suited for the binding of whole cells, yeast, bacteria, microbes and other large structures, since antibodies against them are readily obtained

by either classical techniques and/or cloning (See Section 5.3). The procedure is also well suited, however, for the binding of proteins.

Additionally, the affinity binding systems can also be modified and used in cell sorting and affinity chromatography systems. In such systems, the bound antibody or lectin is dispersed in a system containing the ligate mixed with other molecules or cells, etc. The bound antibody or lectin will preferentially bind the ligate and the bound complexes can then be separated from the mixture by any convenient means, such as centrifugation and decantation, filtration, etc. Subsequently, the bound ligate can be reclaimed from the complex by chemical means.

Furthermore, the metal oxide organophosphorus complexes can also serve as a base to which enzymes can be attached. Such attachment can be direct, or via an antibody intermediate. In either case, the enzyme can retain its activity and can, therefore, be used as catalysts in immobilized reactor systems, either batch-type or continuous flow. In such systems, the immobilized enzymes can be intimately mixed with the appropriate substrates to form the desired products.

5.3. PREPARATION OF MONOCLONAL ANTIBODIES

5.3.1. ANTIBODY PRODUCTION

The initial step in obtaining monoclonal antibodies useful in the compositions and processes described and claimed herein is to produce antibodies specifically directed against the appropriate or desired antigen. This step is accomplished by the immunization of an animal with one or more injections of the specific antigen, e.g., hepatocyte tumor cells, GI tract epithelial cells, etc. The presence of the antigen will cause the animal to develop somatic cells which produce the antibodies.

The somatic cells, and in particular B cells, are utilized in the fusion step. The cells are located primarily in the lymph nodes and spleen of the animal and are removed after an immune response is initiated. In general, the choice of the lymphatic organ used should be made in light of the particular fusion system used although little is known about the factors influencing optimization.

5.3.2. FUSION

The antibody-producing (somatic) cells collected above can be fused with any of the various myeloma lines commercially available. Such lines have been developed from lymphocyte tumor lines specifically for use in hybridoma-producing procedures [See, e.g., G. Kohler and C. Milstein, Eur. J. Immunol. 6 511-519 (1976); M. Shulman, et al., Nature 276: 269-270 (1978)]. These cell strains are highly suited for efficient fusion, cannot produce their own antibodies, and, to facilitate separation, possess enzyme deficiencies which prevent them from growing in selective media capable of supporting hybridoma growth (see discussion of this property in the following section).

Various methods for generating fused hybrids of the somatic cells have been described by a number of authors [See e.g., Kohler and Milstein, Nature, 256: 495-497 (1975) and Gefter, et al., Somatic Cell Genet., 3: 231-236 (1977)]. The methods generally involve mixing the somatic cells and the myeloma cells in a 1:1 to 20:1 proportion (preferably 10:1) in the presence of a fusion promoting agent or agents (e.g., polyethylene glycol). The hybridomas must then be collected and isolated as described below.

5.3.3. ISOLATION OF CLONES AND ANTIBODY DETECTION

Because fusion procedures produce viable hybrids at a very low frequency (e.g., when spleen cells are used only one hybrid is obtained for every 2×10^5 cells) it is essential to

have a means for selecting the fused hybrids from the unfused cells, particularly the unfused myeloma cells. Additionally, a means for detecting the desired antibody-producing hybridomas among the other resulting fused cell hybrids is also necessary.

Generally, this selection is accomplished by culturing the cells in media that support the growth of the hybridomas, but not the myeloma cells which normally would go on dividing indefinitely (the non-fused somatic cells cannot maintain viability in in vitro culture and, thus, do not pose a problem). As stated supra, the myeloma cell strains used possess certain specific enzymatic deficiencies which prevent them from metabolizing various nutrients or, alternatively, make them more sensitive to a particular drug, etc. Of course, the fused somatic cells do not have this deficiency and, thus, a culture medium can be chosen which will permit the growth of the fused hybrids, but not the unfused myeloma cells.

Several weeks are required to culture selectively the fused cell hybrids. During this time, the antibody producing hybrids can be identified by any competent assay method including enzyme-linked immunoassay (ELISA) or radio-immunoassay (RIA) techniques; once selected, these fused cell hybrids can be cloned into antibody-producing cell lines.

Each cell line can then be propagated in one of two ways. In the in vitro method, the cell lines are cultivated in laboratory culture vessels and the antibody, which is contained in the medium, can be harvested by decantation, filtration, or centrifugation. Alternatively, the cells can be cultured in vivo by injecting a sample of the hybridoma into a histocompatible animal of the type that was used to provide the somatic and myeloma cells for the original fusion. The injected animal develops tumors secreting the specific monoclonal antibody produced by the cell hybrid. The body fluids of the animal, especially the serum, can then be tapped

to yield a high concentration of the monoclonal antibody which may be utilized as the ligand in the biologically reactive particle of this invention and as part of the processes in which these particles are utilized, which particles and processes are also described in more detail below.

5.4. USE OF COMPLEXES IN BIOLOGICAL ASSAYS

The complexes of this invention may be used in immunoassays and other binding assays. The most prevalent types of assays used for diagnostic and research purposes are radioimmunoassays, fluoroimmunoassays, enzyme-immunoassays, and non-immune radioassays, based on the principle of competitive binding. Basically, a ligand, such as an antibody as described above in Section 5.3 or specific binding protein, directed against a ligate, such as an antigen, is saturated with an excess of labeled ligate (ligate). [Alternatively, competitive assays may be run with labeled ligand and unlabeled ligate. Non-competitive assays, so-called sandwich assays, are also widely employed.] By the method of this invention, the ligand is coupled to a complex. Examples of labels are radioisotopes: tritium, carbon-14, cobalt-57 and, preferably, iodine-125; fluorometric labels: rhodamine or fluorescein isothiocyanate; and enzymes (generally chosen for the ease with which the enzymatic reaction can be measured): alkaline phosphatase or α-D-galactosidase. If unlabeled ligate is added to ligand along with *ligate, less *ligate will be found in the ligand-ligate complex as the ratio of unlabeled to labeled ligate increases. If the ligand-*ligate complex can be physically separated from *ligate, the amount of unlabeled ligate in a test substance can be determined.

To measure unlabeled ligate, a standard curve must be constructed. This procedure is done by mixing a fixed amount of ligand and *ligate and adding a known amount of unlabeled ligate to each. When the reaction is complete, the ligand-*ligate is separated from *ligate. A graph is then made that

relates the label in the collected ligand-*ligate complex to the amount of added unlabeled ligate. To determine the amount of unlabeled ligate in an experimental sample, an aliquot of the sample is added to the same ligand-*ligate mixture used to obtain the standard curve. The ligand-*ligate complex is collected and the label measured, and the amount of unlabeled ligand is read from the standard curve. This measurement is possible with any sample, no matter how complex, as long as nothing interferes with the ligand-*ligate interaction. By the method of this invention, the ligand-*ligate complex can be separated by any convenient means (such as precipitation, centrifugation, or filtration) from free ligate.

This general methodology can be applied in assays for the measurement of a wide variety of compounds including hormones, pharmacologic agents, vitamins and cofactors, hematological substances, virus antigens, nucleic acids, nucleotides, glycosides and sugars. By way of illustration, the compounds listed in Table I are all measurable by magnetic particle immunoassays and binding assays [See D. Freifelder, Physical Biochemistry: Applications to Biochemistry and Molecular Biology, p. 259, W. H. Freeman and Company, San Francisco (1976)].

TABLE I

SUBSTANCES MEASURABLE IN ASSAYS

Hormones:

Thyroid hormones (tyroxine, triiodothyronine, thyroid binding globulin, thyroid-stimulating hormone, thyroglobulin)
Gastrointestinal hormones (glucagon, gastrin, enteroglucagon, secretin, pancreozymin, vasoactive intestinal peptide, gastric inhibitory peptide, motilin, insulin)
Follicle-stimulating hormone
Leutenizing Hormone
Progesterone
Testosterone
Estriol
Estradiol
Prolactin
Thyrocalcitonin
Parathyroid hormone
gonadotrophin
Human placental lactogen
Posterior pituitary peptides (oxytocin, vasopressin, neurophysin)
Bradykinin
Cortisol
Corticotrophin
Human growth hormone

Pharmacologic agents:

Digoxin
Theophylline
Morphine and opiate alkaloids
Cardiac glycosides
Prostaglandins
Lysergic acid and derivatives
Tetrahydrocannabinol
Barbiturates
Nicotine and metabolic products
Phenothiazines
Amphetamines

Vitamins and cofactors:

D, B_{12}, folic acid, cyclic AMP

Hematological substances:

Fibrinogen, fibrin, and fibrinopeptides
Plasminogen and plasmin
Antihemophilic factor
Prothrombin
Transferring and ferritin
Erythropoietin

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Virus antigens:

Hepatitis antigen	Polio
Herpes simplex	Rabies
Vaccinia	Q fever
Several Group A arboviruses	Psittacosis group

Nucleic acids and nucleotides:

DNA, RNA, cytosine derivatives

5.5. USE OF PARTICLES IN IMMOBILIZED ENZYME SYSTEMS

Enzymes may be coupled to the particles of this invention by the methods described in Section 5.2. They may be used in immobilized enzyme systems, particularly in batch reactors or continuous-flow stirred-tank reactors (CSTR), to facilitate separation of enzyme from product after the reaction has occurred and to permit enzyme reuse and recycling.

In such a system, substrates are contacted with enzyme-coupled particles in a reactor under conditions of pH, temperature and substrate concentration that best promote the reaction. After completion of the reaction the particles are separated from the bulk liquid (which may be a solution or suspension) from which product can be retrieved free of enzyme. The enzyme-coupled complexes can then be reused. Immobilized enzymes (coupled to supports) have been used in a number of important industrial enzymatic reactions, some of which are listed in Table II. The complexes of this invention can be substituted for solid phases previously employed which include glass, ceramics, polyacrylamide, DEAE-cellulose, chitin, porous silica, cellulose beads and alumino-silicates.

TABLE II

INDUSTRIALLY IMPORTANT IMMOBILIZED ENZYME REACTIONS

Enzyme	Reactant/Product
Amylo-glucosidase	Maltose/glucose
Glucose Oxidase	Glucose/gluconic acid
Glucoamylase	Starch/glucose, Dextrin/glucose
-Amylase	Starch/maltose
Invertase	Sucrose/glucose
Glucose isomerase	Glucose/fructose
Lactase	Lactose/glucose
Trypsin	Proteins/amino acids
Aminoacylase	N-acetyl-DL-methionine/ methionine
Lysozyme	Lysis of M. lysodeikticus

5.6. USE OF THE BIOLOGICALLY REACTIVE PARTICLES IN CHROMATOGRAPHY

The ternary complexes of the present invention have broad applicability to the area of chromatographic binding and separations. Because of the structural integrity of the metal oxide/hydroxide core, the coupled complexes can be employed as packing materials for normal and high-pressure applications. The metal oxide complexes resist compression and swelling and provide a high surface area for interaction with solutes while permitting a steady flow of eluent resulting in a low back-pressure. Certain thermally-stable combinations can even be suitable for separations conducted at room temperature or higher, such as gas-liquid chromatography. Particle sizes can be controlled as discussed previously making the products of the invention equally suitable for small-scale analytical or large-scale preparative procedures.

Most beneficially, the methods and organophosphorus-modified metal oxide/bioactive component conjugates of the present invention provide unique surfaces which may be custom-designed to suit a particular application. Substantially hydrophobic surfaces can be introduced onto the surface of the particles for reverse phase chromatographic methods. Conversely, highly polar functional groups present on the exposed surface of the particles lend themselves well to normal phase applications. Proteins, polypeptides, or amino acids coupled to the organophosphorus termini provide multiply-charged sites whose overall charge balance may be adjusted by proper selection of the pH of the medium. The degree of interaction of charged or like solutes may thus be dependent on, and thus controllable by, inter alia, its isoelectronic point or pH. Practically, any charged solute may be manipulable by the above means.

In addition, a method for restoring the activity of these columns may preferably include the following: the deactivated column is incubated with a freshly prepared solution of the organophosphorus compound followed by treatment with the bioactive component. In this manner, exposed sites on the metal oxide surface can be recapped with the phosphorus-containing organic molecule. The active surface of the material can then be rejuvenated by readsorption or bonding of fresh bioactive component.

The process of affinity chromatography enables the efficient isolation of molecules by making use of features unique to the molecule: the ability to recognize or be recognized with a high degree of selectivity by a bioaffinity adsorbent such as an enzyme or antibody and the ability to bind or adsorb thereto. The process of affinity chromatography simply involves placing a selective bioaffinity adsorbent or ligand in contact with a solution containing several kinds of substances including the desired species, the ligate. The ligate is selectively adsorbed to the ligand,

which is coupled to an insoluble support or matrix. The nonbinding species are removed by washing. The ligate is then recovered by eluting with a specific desorbing agent, e.g. a buffer at a pH or ionic strength that will cause detachment of the adsorbed ligate.

By the preferred method of this invention, the metal oxide complexes may be used as the insoluble support to which the ligand is coupled. The particles may be suspended in batch reactors containing the ligate to be isolated. The particles with bound ligate may be separated from the bulk fluid and washed. Finally, the ligate can be recovered from the particle by desorption. The complexes of this invention may be used in a variety of affinity systems exemplified by those listed in Table III. The complexes of this invention can also be substituted for solid phases previously used including, for example, silanized controlled pore glass and cellulose affinity adsorbents, agarose, polyacrylamide gels, and ion-exchange resins.

Also, the products of the invention may be applied to affinity absorption, flocculation methods, membrane filtration, and used as filter aids. The materials of the invention may, for example, be dispersed in a colloidal suspension or even a solution containing a dissolved substance of interest, allowing the bioactive component of the particles to interact with the substrate, and simply collecting the resulting absorbed or bound species.

TABLE III

AFFINITY SYSTEMS

Ligand, immobile entity	Ligate, soluble entity
Inhibitor, cofactor, prosthetic group, polymers substrate	Enzymes; apoenzymes
Enzyme	Polymeric inhibitors
Nucleic acid, single strand	Nucleic acid, complementary strand; enzymes

Hapten; antigen	Antibody
Antibody (IgG)	Proteins; polysaccharides
Monosaccharide; polysaccharide	Lectins; receptors
Lectin	Glycoproteins; receptors
Small target compounds	Binding Proteins
Binding Protein	Small target compounds
Oligonucleotides	Nucleic acid, complementary; proteins

5.7. USE OF THE BIOLOGICALLY REACTIVE PARTICLES IN NUCLEIC ACID HYBRIDIZATION

Nucleic acid hybridization techniques provide a sensitive and efficient method for isolation and/or selection of specific nucleotide sequences. If hybridization is to occur, the single stranded DNA or RNA nucleic acid sequences must be immobilized on filters, such as nitro-cellulose filters or activated cellulose papers. After the filters have been prepared, a radioactive single-stranded DNA or RNA is contacted with the filters and incubated under conditions suitable for hybridization. Complementary single-stranded radioactive DNA or RNA molecules bind to the nucleic acid molecules on the filter, the filter is washed to remove unhybridized material, and the radioactivity is determined.

In a preferred method of the present invention, the complexes may be used to immobilize the single stranded-nucleic acid molecules (either DNA or RNA, and homopolymeric oligonucleotides, including polyuridylic acid, polythymidilic acid, polyadenylic acid and polyguanidylic acid). The complexes may be suspended in a large reaction vessel (batch reactors) containing several species of single-stranded nucleic acids, including the complementary single-stranded nucleic acid molecules to be hybridized to the bound molecules. The complexes with bound complementary nucleic acid molecules may then be separated from the reaction mixture, washed repeatedly, and recovered under appropriate conditions.

The complexes of this invention may be used in several different hybridization procedures. In one method, viral DNA or cDNA clones that are complementary to specific mRNAs can be identified by hybridization selection. Cloned or viral DNAs are denatured, immobilized on the magnetic particles and hybridized to preparations containing total cellular mRNA or other preparations of mRNA. The mRNA can then be recovered from the magnetic particle. The released mRNA can be translated, for example in cell free, protein synthesizing systems or in xenopus oocytes and the translation products identified.

Specific DNA or RNA fragments can also be isolated from genomic and cloned DNA by immobilization of a known probe to the magnetic particles and placing the coupled particle in contact with a mixture of nucleic acid fragments, including the desired species. After hybridization the particles may be magnetically separated from unbound materials, washed, and the hybridized molecules isolated.

5.8. USE OF PARTICLES IN CELL SEPARATION

The complexes of the present invention can be used in cell separation and cell sorting procedures. The complexes can be used to distinguish subpopulations of cells for biochemical, immunological and clinical studies. The complexes can be used to decrease cell settling times and to yield the desired cell population with little or no contamination by other cell types.

In a preferred method of this invention, the complexes may be used as an insoluble support to which a bioaffinity adsorbent or ligand such as an antibody, lectin or fluorescent dye is coupled. The complexes may be suspended in batch reactors containing the cells or cells coupled to a ligate to be isolated. The complexes with bound cells may be separated from the unbound cells by any convenient means and washed. Finally, the bound cells can be recovered from the particle by

desorption. By way of illustration, antibodies (polyclonal or monoclonal antibodies) specific for cell surface constituents at the appropriate dilution can be specifically bound to a subpopulation of cells. The complexes of the invention can be coupled to Staphylococcal protein A. Protein A binds specifically with many classes of immunoglobulin molecules through the Fc domain and does not involve the antibody binding site. A cell population with bound antibodies is brought into contact with the coupled protein A complexes under conditions which best promote protein A-antibody-cell complex formation. After the reaction is completed, the complexes are separated from the other cell types. Finally, the isolated subpopulation of cells can be recovered with a dissociating solvent, such as an anionic detergent. Alternatively antibodies can be bound directly to the complexes of the invention. The antibody binding site remains free to interact with a specific cell population as described above.

Fluorescent dyes conjugated to antibodies can also be used to bind to and isolate a specific cell population. The complexes are coupled to antibodies specific for fluorescein and allowed to interact with the fluoresceinated antibody-cell complex. Labeled cells can then be detected under a fluorescent microscope. The complexes are then magnetically separated from the other cell types.

The complexes of the invention may be used for cell sorting or separation of any biologically important cellular populations for which specific antibodies can be produced. They can be used in a variety of cell separation procedures, including for example the separation of T and B lymphocytes from a mixed population of cells, macrophages and other accessory cells from lymphocyte populations, and to separate bacteria and viruses from mixed populations.

It is apparent that many modifications and variations of the invention set forth hereinabove may be made without departing from the spirit and scope thereof. The specific embodiments described are given by way of example only, and the invention is limited only by the terms of the appended claims.

5.9. USE OF BIOLOGICALLY REACTIVE PARTICLES IN METHODS OF TREATING DISEASE

The biologically reactive particles of this invention may be used in a method for treating a disease utilizing the timed release of therapeutic agents which comprises:

(a) using a combination of metal oxide/hydroxide, organophosphorus compound, and biologically active material, in this case, a therapeutic agent, such that the binding interaction between the organophosphorus compound and the therapeutic agent is stable under low pH but unstable in a relatively alkaline environment, or vice-versa; and

(b) administering said combination to a patient suffering from an ailment which can be alleviated by the timed release of the therapeutic agent.

The therapeutic agents may be any drug or pharmaceutical modality known to a physician such as, but not limited to, an antibiotic, anti-neoplastic agent or cardiovascular-active agents. One of ordinary skill in the art such as a physician would know the effective amount and dosages of the therapeutic agent necessary to treat a particular condition.

6. EXAMPLES

The following examples are illustrative only and are not meant to limit the scope and spirit of the invention.

6.1. PREPARATION OF FUNCTIONALIZED ORGANOPHOSPHORUS MODIFIED ALUMINUM OXIDE/HYDROXIDE

Activated alumina (50 g) was added gradually to a boiling 0.1 M isopropanol solution of 3-carboxypropyl phosphonic acid (200 mL). The resulting slurry was stirred slowly and heated under reflux for 60 min. The reaction mixture was then allowed to cool, and the modified alumina particles were collected by vacuum filtration. The solid was washed with fresh solvent (200 mL) and dried at 110°C for 60 min. If desired a second treatment with an isopropanol solution of methyl phosphonic acid can be carried out to cap substantially all of the active sites on the alumina surface. In any event, the dried modified alumina was washed with an aqueous sodium hydroxide/sodium carbonate buffer (pH 10) containing 20% methanol.

It is most desirable to produce a monomolecular layer of organophosphorus compound over the alumina surface. The amount of phosphonic acid adsorbed may be adjusted by varying its solution concentration, changing the amount of metal oxide/hydroxide used, or varying the reaction time and temperature. A similar procedure may be used to prepare the amino-functionalized organophosphorus modified alumina. Other bifunctionalized organophosphorus compounds are available commercially or can be prepared by means well known in the art.

6.2. PROCEDURE FOR BINDING A DYE TO THE MODIFIED ALUMINA PARTICLES

The aluminum oxide/hydroxide particles modified with a carboxy-functionalized organophosphorus compound obtained from the previous example is suspended in an ethereal solvent and treated with an excess amount of 3-(3-dimethylamino)propyl-1-ethylcarbodiimide at room temperature for 1 h. The slurry is then filtered, washed with fresh solvent, and resuspended in the same solvent. A solution of the dye Cibacron Blue F3G-A

(See compound W in Figure 2) in the same solvent is then added, and the coupling is allowed to take place at room temperature over 1-2 h. The excess dye is removed by filtration and washing with fresh solvent.

The product particle is useful for binding a host of enzymes. For examples of enzymes which bind to sulfonated polyaromatic dyes bound to a dextran support [See, Kopperschlager, G. et al., Advances in Biochemical Engineering, 25:103 (1982)].

CLAIMS:

1. A biologically reactive particle capable of binding substances of biological significance comprising:

(a) a metal oxide/hydroxide core consisting essentially of an oxide/hydroxide of a member selected from the class consisting of groups IB, IIA, IIB, IIIA, IIIB, IVA, IVB, VA, VB, VIB, VIIB, and VIII elements, the Lanthanide series, selenium, silicon, boron, arsenic, thorium, uranium, and tellurium;

(b) an organophosphorus compound selected from the group consisting of phosphonic acid having the formula $RPO(OH)_2$, phosphinic acid having the formula RR'POOH and an ester of phosphoric acid having the formula (RO)(R'O)POOH where R comprises a functionalized carbon-containing group having 1-60 carbon atoms and R' is selected from the group consisting of hydrogen and a functionalized carbon-containing group having 1-60 carbon atoms and wherein the phosphorus-containing group of the organophosphorus compound is bound to the active sites on the surface of the metal oxide/hydroxide core forming a monomolecular layer thereby leaving the functionalized carbon-containing group essentially exposed and oriented away from the core surface; and

(c) a biologically active ligand bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer.

2. The biologically reactive particle of claim 1 wherein the reactive functionality of the functionalized carbon-containing group is selected from the group consisting of substituted and unsubstituted aromatic rings, heterocycles, olefins, polyolefins, alkynes, carboxylic acids, aldehydes, ketones, amines, amides, piperidines, anhydrides,

carbohydrates, thiocyanates, isocyanates, esters, alcohols, nitiles, epoxides, oximes, organosilanes, and sulfur-containing organic compounds.

3. The biologically reactive particle of claim 1 wherein the reactive functionality of the functionalized carbon-containing group is selected from the group consisting of halides, nitrates, phosphates, phosphites, phosphinates, phosphines, phosphinites, phosphonates, phosphonites, sulfates, sulfites, sulfonates, sulfinates, sulfides, sulfoxides, thiols, perhalogenated hydrocarbons, cyanides, and quaternary ammonium salts.

4. The biologically reactive particle claim 1 wherein the biologically active ligand is selected from the group consisting of antibodies, antigens, haptens, enzymes, apoenzymes, enzymatic substrates, enzymatic inhibitors, cofactors, binding proteins, carrier proteins, metalloproteins, compounds bound to these proteins, lectins, monosaccharides, oligosaccharides, polysaccharides, hormones, receptors, repressors, inducers, dyes, nucleic acids, amino acids, oligonucleotides, deoxyribonucleic acids, ribonucleic acids, and fragments thereof.

5. The biologically reactive particle of claim 1 wherein the biologically active ligand is selected from the group consisting of polyacrylamides, latex, dextran, agarose, and ion-exchange material.

6. The biologically reactive particle of claim 4 wherein the biologically active ligand is selected from the group consisting of sulfonated polyaromatic, triazine, naphthalene, and anthraquinone dyes.

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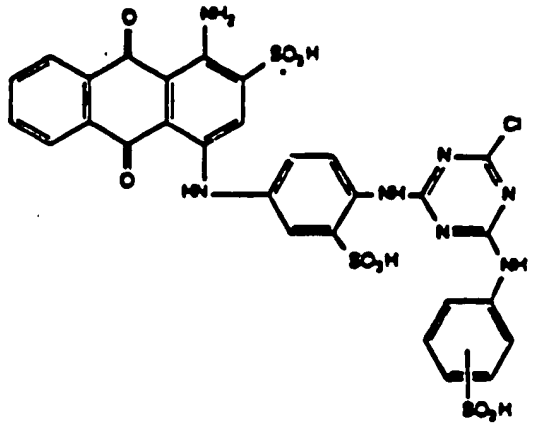
7. The biologically reactive particle of claim 6 wherein the biologically active ligand is a dye having the following structure:



8. The biologically reactive particle of claim 4 wherein the biologically active ligand is selected from the group consisting of anti-throxine, anti- triiodothyronine, anti-thyroid stimulating hormone, anti- thyroid binding globulin, anti-thyroglobulin, anti-digoxin, anti-cortisol, anti-insulin, anti-theophyline, anti-vitamin B_{12}, anti-folate, anti-ferritin, anti-human chorionic gonadotropin, anti-follicle stimulating hormone, anti- leutenizing hormone, anti-progesterone, anti-testosterone, anti-estriol, anti-estradiol, anti-prolactin, anti-human placental lactogen, anti-gastrin, and anti-human growth hormone antibodies.

9. The biologically reactive particle of claim 4 wherein the biologically active ligand is a monoclonal antibody.

10. The biologically reactive particle of claim 4 wherein the biologically active ligand is Protein A.

11. The biologically reactive particle of claim 10 wherein the biologically active ligand further comprises an antibody bound to said Protein A.

12. The biologically reactive particle of claim 1 wherein the biologically active ligand is bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer by a covalent bond.

13. The biologically reactive particle of claim 1 wherein the biologically active ligand is bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer by noncovalent forces.

14. The biologically reactive particle of claim 1 wherein the biologically active ligand is bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer via a conjugative moiety.

15. A method for determining the concentration of a ligate in a solution which comprises:

(a) adding a known amount of labeled ligate to the solution and allowing the resulting solution to react with the biologically reactive particles comprising:

(i) a metal oxide/hydroxide core consisting essentially of an oxide/hydroxide of a member selected from the class consisting of groups IB, IIA, IIB, IIIA, IIIB, IVA, IVB, VA, VI, VIB, VIIB, and VIII elements, the Lanthanide series, selenium, silicon, boron, arsenic, thorium, uranium, and tellurium;

(ii) an organophosphorus compound selected from the group consisting of phosphonic acid having the formula $RPO(OH)_2$, phosphinic acid having the formula RR'POOH and an ester of phosphoric acid

having the formula (RO)(R'O)POOH where R comprises a functionalized carbon-containing group having 1-60 carbon atoms and R' is selected from the group consisting of hydrogen and a functionalized carbon-containing group having 1-60 carbon atoms and wherein the phosphorus-containing group of the organophosphorus compound is bound to the active sites on the surface of the metal oxide/hydroxide core forming a monomolecular layer thereby leaving the functionalized carbon-containing group essentially exposed and oriented away from the core surface; and

(iii) a biologically active ligand bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer whose biologically active ligand is specific for that ligate, so as to form ligand/ligate complexes;

(b) separating the resultant complexes from the reaction mixture of step (a);

(c) measuring the amount of labeled ligate associated with the resultant complexes or remaining free in the reaction mixture; and

(d) comparing the value obtained from step (c) to a corresponding value for the original ligate concentration on a standard curve.

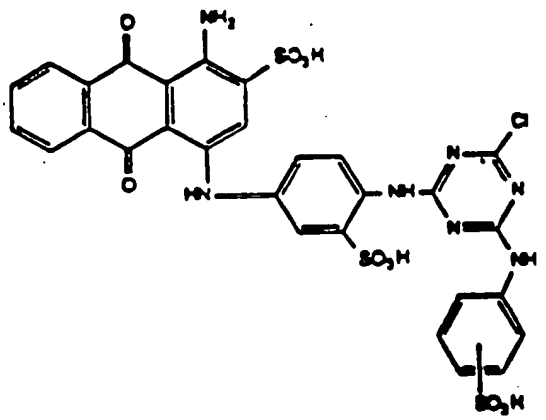
16. The method of claim 15 wherein the biologically active ligand is selected from the group consisting of antibodies, antigens, haptens, enzymes, apoenzymes, enzymatic substrates, enzymatic inhibitors, cofactors, binding proteins, carrier proteins, metalloproteins, compounds bound to these proteins, lectins, monosaccharides, oligosaccharides, polysaccharides, hormones, receptors, repressors, inducers,

dyes, nucleic acids, amino acids, oligonucleotides, deoxyribonucleic acids, ribonucleic acids, and fragments thereof.

17. The method of claim 15 wherein the biologically active ligand is selected form the group consisting of polyacrylamides, latex, dextran, agarose, and ion-exchange material.

18. The method of claim 15 wherein the biologically active ligand is a member selected from the group consisting of sulfonated polyaromatic, triazine, naphthalene, and anthraquinone dyes.

19. The method of claim 15 wherein the biologically active ligand is a dye having the following structure:



20. The method of claim 15 wherein the biologically active ligand is selected from the group consisting of anti-throxine, anti-triiodothyronine, anti-thyroid stimulating hormone, anti-thyroid binding globulin, anti-thyroglobulin, anti-digoxin, anti-cortisol, anti-insulin, anti-theophyline, anti-vitamin B_{12}, anti-folate, anti-ferritin, anti human chorionic gonadotropin, anti-follicle stimulating hormone, anti-leutenizing hormone, anti-progesterone, anti-

testosterone, anti-estriol, anti-estradiol, anti-prolactin, anti-human placental lactogen, anti-gastrin, and anti-human growth hormone antibodies.

21. The method of claim 15 wherein the biologically active ligand is a monoclonal antibody.

22. The method of claim 15 wherein the biologically active ligand is Protein A.

23. The method of claim 15 wherein the biologically active ligand further comprises an antibody bound to said Protein A.

24. The method of claim 15 wherein the biologically active ligand is bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer by a covalent bond.

25. The method of claim 15 wherein the biologically active ligand is bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer by noncovalent forces.

26. The method of claim 15 wherein the biologically active ligand is bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer via a conjugative moiety.

27. A process utilizing an enzymatic reaction which comprises:

(a) contacting the biologically reactive particles of comprising:

(i) a metal oxide/hydroxide core consisting essentially of an oxide/hydroxide of a member selected from the class consisting of groups IB, IIA, IIB, IIIA, IIIB, IVA, IVB, VA, VB, VIB, VIIB, and VIII elements, the Lanthanide series, selenium, silicon, boron, arsenic, thorium, uranium, and tellurium;

(ii) an organophosphorus compound selected from the group consisting of phosphonic acid having the formula $RPO(OH)_2$, phosphinic acid having the formula RR'POOH and an ester of phosphoric acid having the formula (RO)(R'O)POOH where R comprises a functionalized carbon-containing group having 1-60 carbon atoms and R' is selected from the group consisting of hydrogen and a functionalized carbon-containing group having 1-60 carbon atoms and wherein the phosphorus-containing group of the organophosphorus compound is bound to the active sites on the surface of the metal oxide/hydroxide core forming a monomolecular layer thereby leaving the functionalized carbon-containing group essentially exposed and oriented away from the core surface; and

(iii) a biologically active ligand bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer whose biologically active ligand is an enzyme, with a mixture of a reactive substrate contained in an appropriate reaction vessel;

(b) allowing the enzymatic reaction to proceed under suitable conditions; and

(c) removing the biologically reactive particles and

(d) separating the products from the resulting mixture.

28. A process for carrying out nucleic acid hybridization which comprises:

(a) contacting the biologically reactive particles comprising:

(i) a metal oxide/hydroxide core consisting essentially of an oxide/hydroxide of a member selected from the class consisting of groups IB, IIA, IIB, IIIA, IIIB, IVA, IVB, VA, VB, VIB, VIIB, and VIII elements, the Lanthanide series, selenium, silicon, boron, arsenic, thorium, uranium, and tellurium;

(ii) an organophosphorus compound selected from the group consisting of phosphonic acid having the formula $RPO(OH)_2$, phosphinic acid having the formula RR'POOH and an ester of phosphoric acid having the formula (RO)(R'O)POOH where R comprises a functionalized carbon-containing group having 1-60 carbon atoms and R' is selected from the group consisting of hydrogen and a functionalized carbon-containing group having 1-60 carbon atoms and wherein the phosphorus-containing group of the organophosphorus compound is bound to the active sites on the surface of the metal oxide/hydroxide core forming a monomolecular layer thereby leaving the functionalized carbon-containing group essentially exposed and oriented away from the core surface; and

(iii) a biologically active ligand bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer whose biologically active ligand is a nucleic acid, with a solution or suspension of the complementary nucleic acid contained in an appropriate reaction vessel;

(b) allowing the bound nucleic acid to hybridize with its complementary nucleic acid;

(c) separating the particles with complementary nucleic acid molecules bound thereto from the solution or suspension; and

(d) recovering the nucleic acid hybrids from the particles.

29. The process according to claim 28 wherein the biologically active ligand is deoxyribonucleic acid.

30. The process according to claim 28 wherein the biologically active ligand is ribonucleic acid.

31. The process according to claim 28 wherein the biologically active ligand is a homopolymeric oligonucleotide.

32. The process according to claim 31 wherein the homopolymeric oligonucleotide is selected from the group consisting of polyuridylic acid, polythymidylic acid, polyadenylic acid, and polyguadinylic acid.

33. A method for selecting a subpopulation of cells, bacteria, or viruses from a heterogeneous population which comprises:

(a) incubating a solution or suspension of the heterogeneous population with a ligate which binds specifically to the subpopulation of interest;

(b) allowing the resulting solution or suspension to react with the biologically reactive particles comprising:

(i) a metal oxide/hydroxide core consisting essentially of an oxide/hydroxide of a member selected from the class consisting of groups IB, IIA, IIB, IIIA, IIIB, IVA, IVB, VA, VB, VIB, VIIB,

and VIII elements, the Lanthanide series, selenium, silicon, boron, arsenic, thorium, uranium, and tellurium;

(ii) an organophosphorus compound selected from the group consisting of phosphonic acid having the formula $RPO(OH)_2$, phosphinic acid having the formula RR'POOH and an ester of phosphoric acid having the formula (RO)(R'O)POOH where R comprises a functionalized carbon-containing group having 1-60 carbon atoms and R' is selected from the group consisting of hydrogen and a functionalized carbon-containing group having 1-60 carbon atoms and wherein the phosphorus-containing group of the organophosphorus compound is bound to the active sites on the surface of the metal oxide/hydroxide core forming a monomolecular layer thereby leaving the functionalized carbon-containing group essentially exposed and oriented away from the core surface; and

(iii) a biologically active ligand bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer whose biologically active ligand is specific for the added ligate, for a length of time sufficient to form ligand/ligate aggregates;

(c) separating the aggregates from the solution or suspension; and

(d) recovering the desired subpopulation from said aggregates.

34. The method of claim 33 wherein the biologically active ligand is an antibody.

35. The method of claim 33 wherein the biologically active ligand is Protein A.

36. The method of claim 33 wherein the biologically active ligand is a dye.

37. The method of claim 33 wherein the biologically active ligand is a lectin.

38. The method of claim 33 wherein the biologically active ligand is heparin.

39. The method of claim 33 wherein the biologically active ligand is an antibody specific for fluorescein and the added ligate is fluorescein.

40. The method of claim 35 wherein the added ligate is an antibody.

41. A method for selecting a subpopulation of cells, bacteria, or viruses from a heterogeneous population which comprises:

(a) allowing a solution or suspension of the heterogeneous population to react with the biologically reactive particles comprising:

(i) a metal oxide/hydroxide core consisting essentially of an oxide/hydroxide of a member selected from the class consisting of groups IB, IIA, IIB, IIIA, IIIB, IVA, IVB, VA, VB, VIB, VIIB, and VIII elements, the Lanthanide series, selenium, silicon, boron, arsenic, thorium, uranium, and tellurium;

(ii) an organophosphorus compound selected from the group consisting of phosphonic acid having the formula $RPO(OH)_2$, phosphinic acid having the formula RR'POOH and an ester of phosphoric acid having the formula (RO)(R'O)POOH where R comprises a functionalized carbon-containing group having

1-60 carbon atoms and R' is selected from the group consisting of hydrogen and a functionalized carbon-containing group having 1-60 carbon atoms and wherein the phosphorus-containing group of the organophosphorus compound is bound to the active sites on the surface of the metal oxide/hydroxide core forming a monomolecular layer thereby leaving the functionalized carbon-containing group essentially exposed and oriented away from the core surface; and

(iii) a biologically active ligand bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer whose biologically active ligand is an antibody specific for the subpopulation of interest, for a length of time sufficient to form coupled complexes;

(b) separating the coupled complexes from the solution or suspension; and

(c) recovering the desired subpopulation from the isolated coupled complexes.

42. A packing material suitable for chromatographic applications which comprises:

(a) a metal oxide/hydroxide core consisting essentially of an oxide/hydroxide of a member selected from the class consisting of groups IB, IIA, IIB, IIIA, IIIB, IVA, IVB, VA, VB, VIB, VIIB, and VIII elements, the Lanthanide series, selenium, silicon, boron, arsenic, thorium, uranium, and tellurium;

(b) an organophosphorus compound selected from the group consisting of phosphonic acid having the formula $RPO(OH)_2$, phosphinic acid having the formula RR'POOH and an ester of phosphoric acid having the formula (RO)(R'O)POOH where R comprises a functionalized carbon-containing group having 1-60 carbon atoms and R'

is selected form the group consisting of hydrogen and a functionalized carbon-containing group having 1-60 carbon atoms and wherein the phosphorus-containing group of the organophosphorus compound is bound to the active sites on the surface of the metal oxide/hydroxide core forming a monomolecular layer and leaving the functionalized carbon-containing group essentially exposed and oriented away from the core surface; and

(c) a biologically active ligand bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer.

43. The packing material of claim 41 wherein the reactive functionality of the functionalized carbon-containing group is selected from the group consisting of substituted and unsubstituted aromatic rings, heterocycles, olefins, polyolefins, alkynes, carboxylic acids, aldehydes, ketones, amines, amides, piperidines, anhydrides, carbohydrates, thiocyanates, isocyanates, esters, alcohols, nitiles, epoxides, oximes, organosilanes, and sulfur-containing organic compounds.

44. The packing material of claim 41 wherein the reactive functionality of the functionalized carbon-containing group is selected from the group consisting of halides, nitrates, phosphates, phosphites, phosphinates, phosphines, phosphinites, phosphonates, phosphonites, sulfates, sulfites, sulfonates, sulfinates, sulfides, sulfoxides, thiols, perhalogenated hydrocarbons, cyanides, and quaternary ammonium salts.

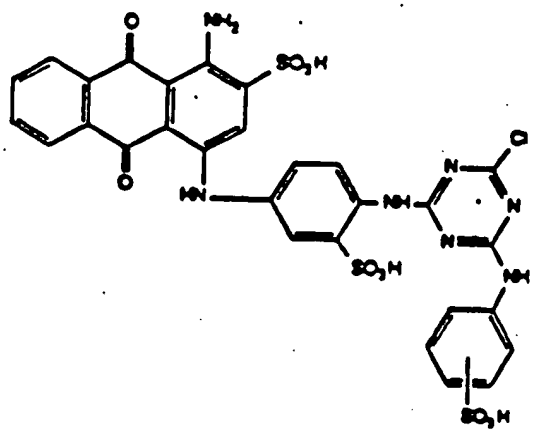
45. The packing material of claim 41 wherein the biologically active ligand is selected from the group consisting of antibodies, antigens, haptens, enzymes,

apoenzymes, enzymatic substrates, enzymatic inhibitors, cofactors, binding proteins, carrier proteins, metalloproteins, compounds bound to these proteins, lectins, monosaccharides, oligosaccharides, polysaccharides, hormones, receptors, repressors, inducers, dyes, nucleic acids, amino acids, oligonucleotides, deoxyribonucleic acids, ribonucleic acids, and fragments thereof.

46. The packing material of claim 41 wherein the biologically active ligand is selected from the group consisting of polyacrylamides, latex, dextran, agarose, and ion-exchange material.

47. The packing material of claim 41 wherein the biologically active ligand is selected from the group consisting of sulfonated polyaromatic, triazine, naphthalene, and anthraquinone dyes.

48. The packing material of claim 41 wherein the biologically active ligand is a dye having the following structure:



49. The packing material of claim 41 wherein the biologically active ligand is selected from the group consisting of anti-throxine, anti-triiodothyronine, anti-

thyroid stimulating hormone, anti-thyroid binding globulin, anti-thyroglobulin, anti-digoxin, anti-cortisol, anti-insulin, anti-theophyline, anti-vitamin B_{12}, anti-folate, anti-ferritin, anti-human chorionic gonadotropin, anti-follicle stimulating hormone, anti-leutenizing hormone, anti-progesterone, anti-testosterone, anti-estriol, anti-estradiol, anti-prolactin, anti-human placental lactogen, anti-gastrin, and anti-human growth hormone antibodies.

50 The packing material of claim 41 wherein the biologically active ligand is a monoclonal antibody.

51. The packing material of claim 41 wherein the biologically active ligand is Protein A.

52. The packing material of claim 51 wherein the biologically active ligand further comprises an antibody bound to said Protein A.

53. The packing material of claim 41 wherein the biologically active ligand is bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer by a covalent bond.

54. The packing material of claim 41 wherein the biologically active ligand is bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer by noncovalent forces.

55. The packing material of claim 41 wherein the biologically active ligand is bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer via a conjugative moiety.

56. An affinity chromatography method for isolating a ligate from a mixture which comprises:

(a) passing a solution or suspension of the mixture through a column filled with the packing material comprises:

(i) a metal oxide/hydroxide core consisting essentially of an oxide/hydroxide of a member selected from the class consisting of groups IB, IIA, IIB, IIIA, IIIB, IVA, IVB, VA, VB, VIB, VIIB, and VIII elements, the Lanthanide series, selenium, silicon, boron, arsenic, thorium, uranium, and tellurium;

(ii) an organophosphorus compound selected from the group consisting of phosphonic acid having the formula $RPO(OH)_2$, phosphinic acid having the formula RR'POOH and an ester of phosphoric acid having the formula (RO)(R'O)POOH where R comprises a functionalized carbon-containing group having 1-60 carbon atoms and R' is selected form the group consisting of hydrogen and a functionalized carbon-containing group having 1-60 carbon atoms and wherein the phosphorus-containing group of the organophosphorus compound is bound to the active sites on the surface of the metal oxide/hydroxide core forming a monomolecular layer and leaving the functionalized carbon-containing group essentially exposed and oriented away from the core surface; and

(iii) a biologically active ligand bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer whose biologically active ligand is specific for the ligate of interest;

(b) washing the column with a solvent suitable for removing unbound substrates;

(c) introducing onto the column a desorbing eluent capable of reversing the ligand/ligate interaction; and

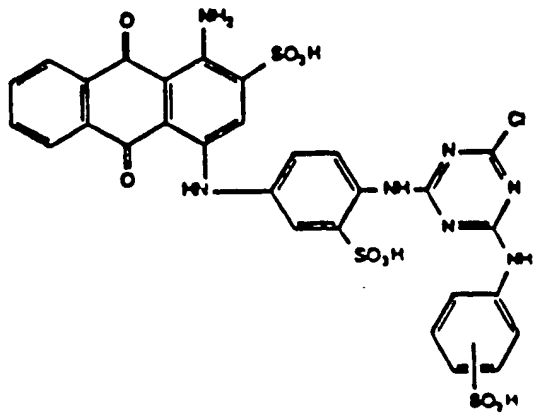
(d) collecting the fractions containing the desired isolated ligate.

57. The method of claim 56 wherein the biologically active ligand is selected from the group consisting of antibodies, antigens, haptens, enzymes, apoenzymes, enzymatic substrates, enzymatic inhibitors, cofactors, binding proteins, carrier proteins, metalloproteins, compounds bound to these proteins, lectins, monosaccharides, oligosaccharides, polysaccharides, hormones, receptors, repressors, inducers, dyes, nucleic acids, amino acids, oligonucleotides, deoxyribonucleic acids, ribonucleic acids, and fragments thereof.

58. The method of claim 56 wherein the biologically active ligand is a member selected from the group consisting of polyacrylamides, latex, dextran, agarose, and ion-exchange material.

59. The method of claim 56 wherein the biologically active ligand is a member selected from the group consisting of sulfonated polyaromatic, triazine, naphthalene, and anthraquinone dyes.

60. The method of claim 59 wherein the biologically active ligand is a dye having the following structure:



61. The method of claim 56 wherein the biologically active ligand is a member selected from the group consisting of anti-throxine, anti-triiodothyronine, anti-thyroid stimulating hormone, anti-thyroid binding globulin, anti-thyroglobulin, anti-digoxin, anti-cortisol, anti-insulin, anti-theophyline, anti vitamin B_{12}, anti-folate, anti-ferritin, anti-human chorionic gonadotropin, anti-follicle stimulating hormone, anti-leutenizing hormone, anti-progesterone, anti- testosterone, anti-estriol, anti-estradiol, anti-prolactin, anti human placental lactogen, anti-gastrin, and anti-human growth hormone antibodies.

62. The method of claim 56 wherein the biologically active ligand is a monoclonal antibody.

63. The method of claim 56 wherein the biologically active ligand is Protein A.

64. The method of claim 63 wherein the biologically active ligand further comprises an antibody bound to said Protein A.

65. A high performance liquid chromatography method employing the packing material comprising:

(a) a metal oxide/hydroxide core consisting essentially of an oxide/hydroxide of a member selected from the class consisting of groups IB, IIA, IIB, IIIA, IIIB, IVA, IVB, VA, VB, VIB, VIIB, and VIII elements, the Lanthanide series, selenium, silicon, boron, arsenic, thorium, uranium, and tellurium;

(b) an organophosphorus compound selected from the group consisting of phosphonic acid having the formula $RPO(OH)_2$, phosphinic acid having the formula RR'POOH and an ester of phosphoric acid having the formula (RO)(R'O)POOH where R comprises a functionalized

carbon-containing group having 1-60 carbon atoms and R' is selected form the group consisting of hydrogen and a functionalized carbon-containing group having 1-60 carbon atoms and wherein the phosphorus-containing group of the organophosphorus compound is bound to the active sites on the surface of the metal oxide/hydroxide core forming a monomolecular layer and leaving the functionalized carbon-containing group essentially exposed and oriented away from the core surface; and

(c) a biologically active ligand bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer.

66. A gas-liquid chromatography method employing the packing material comprising:

(a) a metal oxide/hydroxide core consisting essentially of an oxide/hydroxide of a member selected from the class consisting of groups IB, IIA, IIB, IIIA, IIIB, IVA, IVB, VA, VB, VIB, VIIB, and VIII elements, the Lanthanide series, selenium, silicon, boron, arsenic, thorium, uranium, and tellurium;

(b) an organophosphorus compound selected from the group consisting of phosphonic acid having the formula $RPO(OH)_2$, phosphinic acid having the formula RR'POOH and an ester of phosphoric acid having the formula (RO)(R'O)POOH where R comprises a functionalized carbon-containing group having 1-60 carbon atoms and R' is selected form the group consisting of hydrogen and a functionalized carbon-containing group having 1-60 carbon atoms and wherein the phosphorus-containing group of the organophosphorus compound is bound to the active sites on the surface of the metal oxide/hydroxide core forming a monomolecular layer and

leaving the functionalized carbon-containing group essentially exposed and oriented away from the core surface; and

(c) a biologically active ligand bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer.

67. A method for regenerating a column filled with the packing material comprising:

(i) a metal oxide/hydroxide core consisting essentially of an oxide/hydroxide of a member selected from the class consisting of groups IB, IIA, IIB, IIIA, IIIB, IVA, IVB, VA, VB, VIB, VIIB, and VIII elements, the Lanthanide series, selenium, silicon, boron, arsenic, thorium, uranium, and tellurium;

(ii) an organophosphorus compound selected from the group consisting of phosphonic acid having the formula $RPO(OH)_2$, phosphinic acid having the formula RR'POOH and an ester of phosphoric acid having the formula (RO)(R'O)POOH where R comprises a functionalized carbon-containing group having 1-60 carbon atoms and R' is selected form the group consisting of hydrogen and a functionalized carbon-containing group having 1-60 carbon atoms and wherein the phosphorus-containing group of the organophosphorus compound is bound to the active sites on the surface of the metal oxide/hydroxide core forming a monomolecular layer and leaving the functionalized carbon-containing group essentially exposed and oriented away from the core surface; and

(iii) a biologically active ligand bound to the functionalized carbon-containing group of said organophosphorus monomolecular layer which comprises:

(a) incubating the column with a solution of the original organophosphorus compound used in the packing material for a length of time sufficient to "recap" any exposed active sites on the surface of the metal oxide/hydroxide core;

(b) washing the column with a solvent suitable for removing unbound excess organophosphorus compound;

(c) treating the monomolecular layer with a conjugative moiety, if necessary;

(d) incubating the column with a solution of the desired biologically active ligand for a length of time sufficient for binding to the functionalized carbon-containing group of the organophosphorus monomolecular layer; and

(e) washing the column with a solvent suitable for removing unbound excess ligand.

68. A method of claim 65 wherein the pH ranges from a pH of 1-14.

69. A method for treating a disease utilizing the timed release of therapeutic agents which comprises:

(a) using a composition comprising a metal oxide/hydroxide, organophosphorus compound, a therapeutic agent, such that the binding interaction between the organophosphorus compound and the therapeutic agent is stable under low pH but unstable in a relatively alkaline environment, or vice-versa; and

(b) administering said composition to a patient suffering from an ailment which can be alleviated by the timed release of said therapeutic agent.

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